

Report

Potential Disruption of Pollination in a Sexually Deceptive Orchid by Climatic Change

Karen M. Robbirt,^{1,2} David L. Roberts,^{1,3}
Michael J. Hutchings,⁴ and Anthony J. Davy^{2,*}

¹Royal Botanic Gardens, Kew, Surrey TW9 3AB, UK

²Centre for Ecology, Evolution, and Conservation, School of Biological Sciences, University of East Anglia, Norwich, Norfolk NR4 7TJ, UK

³Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, Marlowe Building, University of Kent, Canterbury, Kent CT2 7NR, UK

⁴School of Life Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, UK

Summary

Warmer springs advance many phenological events, including flowering time in plants and the flight time of insects [1]. Pollination by insects, an ecosystem service of immense economic and conservation importance [2], depends on synchrony between insect activity and flowering time. If plants and their pollinators show different phenological responses to climate warming, pollination could fail. Information about the effects of warming on specific plant-insect mutualisms is difficult to obtain from complex pollination networks [3]. In contrast, the extraordinarily specific deceptions evolved by orchids [4] that attract a very narrow range of pollinators allow direct examination of the potential for climatic warming to disrupt synchrony. Here we show that a sexually deceptive orchid and the solitary bee on which it depends for pollination will diverge in phenology with increasing spring temperature. Male bees inadvertently pollinate the orchid flowers during pseudocopulation. Analysis of museum specimens (1893–2007) and recent field-based records (1975–2009) showed that flight date of the solitary bee *Andrena nigroaenea* is advanced more by higher temperatures than is flowering date in the deceptive orchid *Ophrys sphegodes*. Male bees emerged slightly earlier than females, which attract male copulatory attentions away from the deceptive flowers. Warming by as little as 2°C increased both the probability of male flight and the proportion of females flying in the bee population before orchid flowering; this would reduce the frequency of pseudocopulation and thus lower pollination success rate in the orchid. Our results demonstrate a significant potential for coevolved plant-pollinator relationships to be disrupted by climatic warming.

Results

Using herbarium and field records collected over 159 years, we have already shown that peak flowering time of *Ophrys sphegodes* (early spider orchid) in Britain is advanced by warmer springs [5]. *O. sphegodes* is pollinated almost exclusively by the solitary mining bee *Andrena nigroaenea*, one of the first bees to emerge in spring [6]. We hypothesized that flight

date of *A. nigroaenea* would also be advanced by warmer springs. Although direct observations of flight date are available only for recent years, a further hypothesis was that they could be used to test for a relationship with mean spring temperature; a significant relationship would validate the use of specimen-based records held in various collections that have the potential to provide a longer perspective on the effects of climate on flight date. Museum specimens also allow male and female bees to be distinguished and their phenological responses to climate to be compared. This is important because female bees are expected to be superior to orchid flowers in competing for the copulatory attentions of male bees [7, 8], even though orchids may use stronger floral odor signals to attract pollinators [9].

Bees Fly Earlier in Warmer Springs

We examined recent (1975–2009) field records of the flight date of *A. nigroaenea* in the British Isles from the database of the Bees, Wasps, and Ants Recording Society (BWARS) and established relationships with spring temperature in the Central England Temperature (CET) record; we also looked for effects of latitude and longitude on flight date (see the [Experimental Procedures](#) for details). Flight date was significantly correlated with temperature averaged over a range of 1-month and 3-month periods. Means for March–May and February–April provided the strongest bases for comparison (Table 1). Flight date was earlier in years with warmer springs, advancing by a mean of 7.4 days °C⁻¹ increase in mean spring (March–May) temperature (Figure 1A). Adjustment of the regression for latitude and longitude of the records made no significant difference to this relationship (7.7 days °C⁻¹).

We then investigated the same relationships with temperature in the records of 357 museum specimens of *A. nigroaenea* held at the Natural History Museum, London, and the Oxford University Museum of Natural History. Two hundred and eight specimens were male bees and 149 were females. On average, museum specimens were flying 3.3 days earlier than those in the BWARS database; they showed a significant advance of flight time with mean spring temperature (11.5 days °C⁻¹ mean March–May temperature). This was not significantly different from the advance seen in the BWARS data. Again, adjustment for latitude and longitude of collection did not significantly alter the advancement (11.3 days °C⁻¹).

Temperature Responses of Male and Female Bees Are Different

Having established the utility of museum specimens for validating flight date responses to temperature over the longer time span, we analyzed the responses of male and female bees to temperature separately. Males flew on average 4.05 ± 2.57 days earlier than female bees (not significant). Male flight date responded most strongly to mean temperatures earlier in the season (Figure 1B) than did female flight date (Figure 1C): the best overall predictors for male and female flight date were 3-month mean temperatures for February–April and March–May, respectively (Table 1). Female bees, however, were more responsive to temperature (flight date 15.6 days

*Correspondence: a.davy@uea.ac.uk



Table 1. Correlations of Flight Date of *Andrena nigroaenea*, and Flowering Date of *Ophrys sphegodes*, with Mean Temperature

	Flight Date of <i>Andrena</i>			Flowering Date of <i>Ophrys</i>
	BWARS Data (1975–2009)	Museum Data (1893–2007)		Herbarium and Field Data (1848–2006)
Period of Temperature Mean	n = 2980	Males (n = 208)	Females (n = 149)	n = 102
1-Month Means				
June	0.030 ⁽⁴⁾	0.145*	−0.168*	−0.146
May	−0.125** ⁽⁵⁾	0.039	−0.204** ⁽³⁾	−0.308**
April	−0.147** ⁽²⁾	−0.135*	−0.254** ⁽¹⁾	−0.197*
March	−0.159** ⁽³⁾	−0.272** ⁽²⁾	−0.245** ⁽²⁾	−0.430**
February	−0.186** ⁽¹⁾	−0.354** ⁽¹⁾	−0.171*	−0.215*
January	−0.097**	−0.243** ⁽³⁾	−0.113	−0.114
3-Month Means				
March–May	−0.210**	−0.237**	−0.408**	−0.477**
February–April	−0.216**	−0.396**	−0.309**	−0.381**
December–February	−0.144**	−0.306**	−0.154*	−0.159
Maximum r^2 for multiple regression	0.054	0.169	0.167	0.232

Temperatures were calculated for different periods: monthly from January to June, and three 3-month periods. Also shown is the maximum r^2 for stepwise forward multiple regressions of flight date and flowering date against individual monthly mean temperatures. ** $p < 0.01$, * $p < 0.05$ (one tailed). Superscripted numbers in parentheses indicate the order of inclusion of monthly mean temperatures in stepwise (forward) regression.

earlier $^{\circ}\text{C}^{-1}$ increase in mean March–May temperature) than males (flight date 9.2 days earlier $^{\circ}\text{C}^{-1}$ increase in mean February–April temperature).

Flight Dates and Flowering Dates Respond Differently to Spring Warming

The crucial comparison is between mean flight dates of *A. nigroaenea*, the pollinator, and the peak flowering date of *O. sphegodes*, the recipient of this pollinator activity (Table 1). Peak flowering date was estimated using 102 herbarium and field records of flowering time between 1848 and 2006 from Robbirt et al. [5]. Advancement of flowering was 6.4 days $^{\circ}\text{C}^{-1}$ mean March–May temperature (Figure 2). On average, the flight date of male bees was significantly earlier (8.0 days, $t = 4.1$, $p < 0.0001$) than the peak flowering date of the orchid, but the female flight date was not (4.0 days, $t = 1.67$, $p = 0.096$).

Peak orchid flowering time was predicted for each year for which there was a bee record, using the regression model (Figure 2), and related to recorded bee activity. Male bees held in museum collections were collected in 63 different years between 1893 and 2007; the probability of the mean collection date of male bees preceding the predicted peak flowering date of *O. sphegodes* increased as mean spring temperature increased (Figure 3A). In the 43 years in which both male and female specimens were collected, the probability of a bee collected on or before the predicted peak flowering date being female was greater in years with warmer springs (Figure 3B). Furthermore, in the 38 years in which bees were collected before predicted flowering date, the probability of any individual bee being female was greater in years with warmer springs (Figure 3C). Therefore, after warmer springs, not only would more male bees have been flying prior to the flowering of the orchid, but there would also have been more female bees attracting the copulatory services of male bees at the expense of the orchids.

Discussion

This is, to our knowledge, the first clear example, supported by long-term data, of the potential for climate change to disrupt critical relationships between species. Long-term

phenological trends in data sets [10, 11] are easily obscured by interannual variation [12]. Furthermore, most data sets record the commencement of seasonal events, rather than central (peak) values of the events, which are more evolutionarily significant and reliable for examining phenological trends [13]. The approach used in this study overcomes both of these problems. Recently, specimen-based records held in herbaria and museum collections have been recognized as having considerable unexploited potential as a source of reliable long-term phenological data on many species [14–18]. For example, the average collection date of herbarium specimens of a plant species in any given year accurately reflects its peak flowering time in that year [16]. Such data have been shown to be valid proxies for field observations of peak flowering date [5], and this study similarly validates the use of museum data as proxies for peak date of flight in bees. It is probable that other types of biological specimens can be used to examine other phenological events in a similar way.

Differing phenological responses of individual species to climate warming may lead to the breakdown of coevolved and incidental interactions between species [19–22], because they respond either to different cues or to the same cues at different rates [23]. Highly coevolved insect pollination systems, in which cross-pollination is entirely reliant on one or two pollinator species [24], could be particularly susceptible to disruption by climatic warming. Many orchid species have evolved visual and olfactory characteristics that attract male insects to attempt copulation with the flower, resulting in inadvertent pollination as a result of sexual deceit. As seed production in orchids is generally pollinator limited [25], divergence between the flight date of the insects that are the subject of deception and the flowering time of the orchids could dramatically reduce orchid fitness.

Coevolved specialist relationships, such as the pseudocopulatory one between *O. sphegodes* and *A. nigroaenea*, are particularly vulnerable to disruption caused by climate change. Pollinator interactions generally would be less at risk: where both plants and insects have the option of numerous mutualist partner species, differing species responses to climate change would be more likely to preserve alternative relationships for achieving pollination. However, our relatively simple

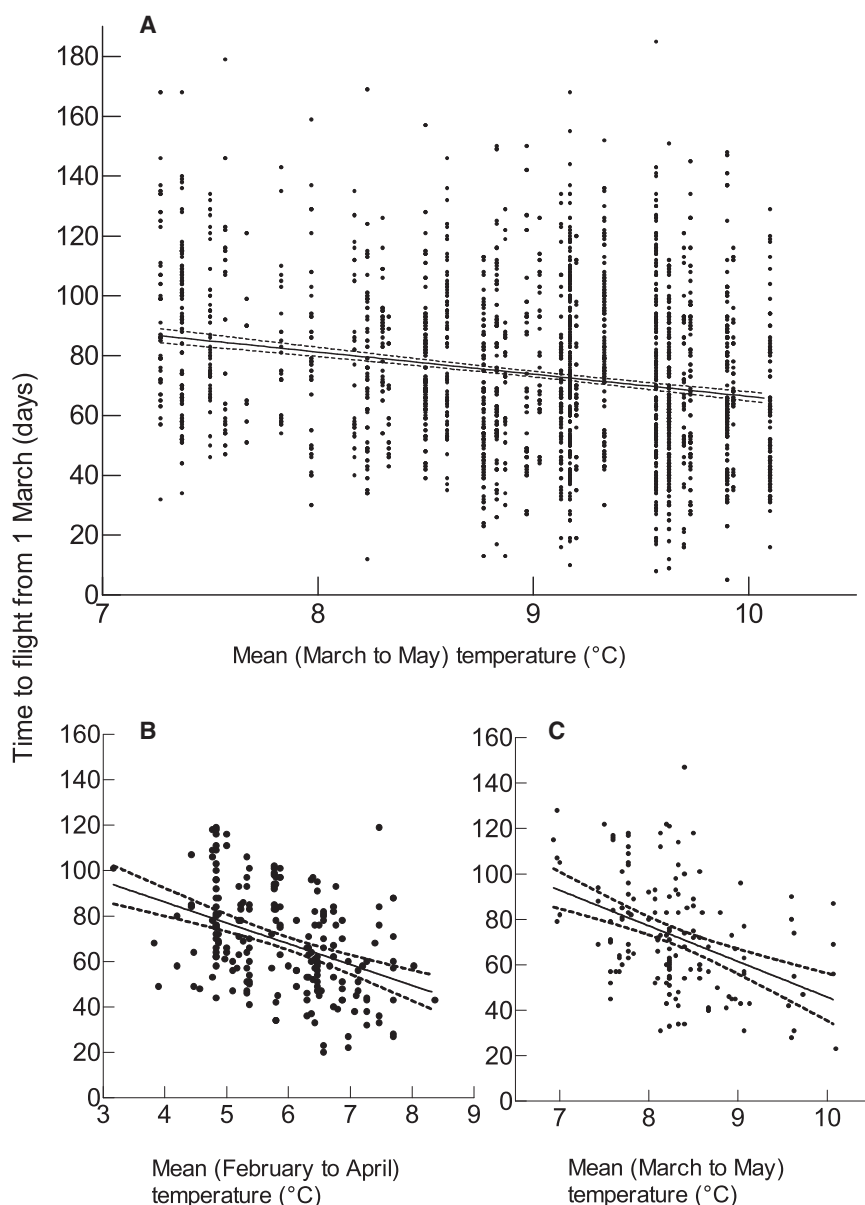


Figure 1. Relationships between Flight Date for *Andrena nigroaenea* and Mean Spring Temperature

Relationships between flight date for *A. nigroaenea* (days after March 1) and mean spring temperature.

(A) Data from BWARS, 1975–2009, in relation to mean March–May temperature ($y = 140.6 - 7.419x$, $r^2 = 0.044$, $p < 0.0001$, $n = 2980$).

(B) Data from male museum specimens, 1893–2004, in relation to mean February–April temperature ($y = 122.8 - 9.168x$, $r^2 = 0.157$, $p < 0.0001$, $n = 208$).

(C) Data from female museum specimens, 1900–2007, in relation to mean March–May temperature ($y = 202.3 - 15.64x$, $r^2 = 0.167$, $p < 0.0001$, $n = 149$).

Garden, Kew; and second, field observations made between 1975 and 2006 on a population of *O. sphegodes* at Castle Hill National Nature Reserve [26].

Flight Date

Recent field records of flight time of *A. nigroaenea* in the British Isles, supplied by the BWARS, were examined to validate the use of museum-specimen records collected over a much longer time span. Sex was determined for almost all of the museum specimens. Records were screened for adequate dating and legibility; geographical concordance with the CET meteorological record; adequate location data; independence; for museum data, reliable sex determination; and, for BWARS, the 1975–2009 period to avoid a highly skewed temporal distribution. A total of 2,980 (81%) of the 3,696 BWARS records were usable. The museum data comprised 848 specimens of *A. nigroaenea* held at the Natural History Museum, London (British Museum; 625 specimens), and Oxford University Museum of Natural History (223 specimens). The final museum data set comprised 357 (42%) specimens, with at least one record for each of 81 years within a 115-year period from 1893 to 2007.

Climatic Data

Mean monthly CET records for the 1848–2010 period [27] were obtained from the UK Meteorological Office (<http://hadobs.metoffice.com/hadcet/cetml1659on.dat>). This is the only complete

climate record available for the years during which the museum records and BWARS field data were collected. Data for central England are drawn from a triangular area bordered at its apices by Bristol, Preston, and London [27].

Analysis

The orchid flowers in late April or May, and flying period for the bee starts before May and continues throughout the flowering time of the orchid. Flowering dates and flight dates were analyzed to determine whether there were correlations with (1) mean individual monthly temperatures in the months December–May and (2) successive 3-month temperature means (December–February, February–April, March–May). Relationships with individual months were also examined using stepwise (forward) multiple regression, and those over longer periods using linear regression. Similarly, we looked for relationships between latitude and longitude on flight or flowering time, using the centroid of the Watsonian vice-county of collection for each specimen. The sensitivity of temperature responses to geographic location was examined using linear regression of residuals after controlling for latitude and longitude.

Acknowledgments

We thank Mark Spencer and David Notton at the Natural History Museum (British Museum) and James Hogan at the Oxford University Museum of

two-species system of an orchid and its pollinating bee has proved amenable to a thorough examination of the potential for climate change to disrupt highly coevolved relationships between species. It provides strong evidence that disruption is very likely to occur. It raises further concerns for the conservation of what is already an endangered species of orchid [26], as it does not appear to have alternative pollinators whose phenology might respond differently to climate change. It also demonstrates that the resources afforded by museum collections have a wealth of untapped value that may be exploited by researchers seeking evidence of the likelihood of phenological disruptions of coevolved interactions in the face of a changing climate.

Experimental Procedures

Flowering Date

Data for peak flowering time of *O. sphegodes* were available from two sources: first, 77 fully dated herbarium specimens collected between 1848 and 1958 and stored in the herbaria of the British Museum and the Royal Botanic

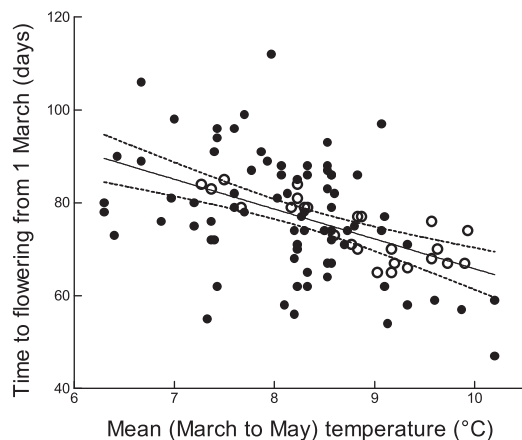


Figure 2. Relationship between Flowering Date of *Ophrys sphegodes* and Mean Spring Temperature

Relationship between flowering date of *O. sphegodes* (days after March 1) and mean spring (March–May) temperature derived from combined herbarium and field data, 1848–2006 ($y = 130.0 - 6.423x$, $r^2 = 0.23$, $p < 0.0001$, $n = 102$). ●, herbarium specimens; ○, field records.

Natural History for access to the botanical and entomological collections. Malcolm Penn (Natural History Museum) calculated the latitude and longitude coordinates of the vice-county centroids. The BWARS very kindly provided access to their extensive observational field data of *Andrena*. We also thank Andrew Bourke and Robert Paxton for discussions on the biology of *Andrena*. K.M.R. was funded by the Swiss Orchid Foundation and Royal Botanic Gardens, Kew.

Received: September 8, 2014

Revised: October 9, 2014

Accepted: October 13, 2014

Published: November 6, 2014

References

- Peñuelas, J., and Filella, I. (2001). Phenology. Responses to a warming world. *Science* 294, 793–795.
- Kearns, C.A., Inouye, D.W., and Waser, N.M. (1998). Endangered mutualisms: the conservation of plant–pollinator interactions. *Annu. Rev. Ecol. Syst.* 29, 83–112.
- Burkle, L.A., and Alarcón, R. (2011). The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *Am. J. Bot.* 98, 528–538.
- Vereecken, N.J., Dafni, A., and Cozzolino, S. (2010). Pollination syndromes in Mediterranean orchids - implications for speciation, taxonomy and conservation. *Bot. Rev.* 76, 220–240.
- Robbirt, K.M., Davy, A.J., Hutchings, M.J., and Roberts, D.L. (2011). Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid *Ophrys sphegodes*. *J. Ecol.* 99, 235–241.
- Archer, M. (2004). BWARS: Bees, Wasps and Ants Recording Society: Members' Handbook (Huntingdon: Centre for Ecology and Hydrology).
- Borg-Karlson, A.-K. (1990). Chemical and ethological studies of pollination in the genus *Ophrys* (Orchidaceae). *Phytochemistry* 29, 1359–1387.
- Peakall, R. (1990). Responses of male *Zaspilothynnus trilobatus* Turner wasps to females and the sexually deceptive orchid it pollinates. *Funct. Ecol.* 4, 159–167.
- Schiestl, F.P. (2004). Floral evolution and pollinator mate choice in a sexually deceptive orchid. *J. Evol. Biol.* 17, 67–75.
- Sparks, T.H. (2007). Lateral thinking on data to identify climate impacts. *Trends Ecol. Evol.* 22, 169–171.
- Magurran, A.E., Baillie, S.R., Buckland, S.T., Dick, J.M., Elston, D.A., Scott, E.M., Smith, R.I., Somerfield, P.J., and Watt, A.D. (2010). Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends Ecol. Evol.* 25, 574–582.

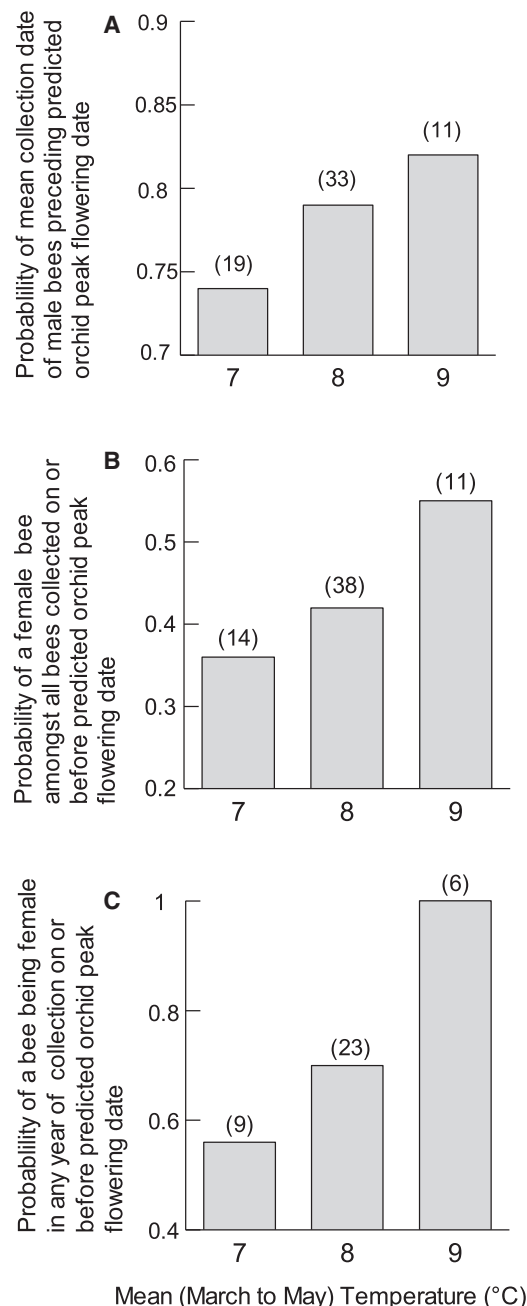


Figure 3. Effects of Mean Spring Temperature on Flight Date of *Andrena nigroaenea* Relative to the Predicted Flowering Date of *Ophrys sphegodes*

Effects of mean spring (March–May) temperature on flight date of *A. nigroaenea* relative to the predicted flowering date of *O. sphegodes*. (A) Fraction of years in which the mean collection date of male bees preceded the predicted peak flowering date (based on 63 years between 1893 and 2007 in which male bees in museum collections had been collected).

(B) Proportion of females among bees collected on or before the predicted flowering date, in the 43 years in which both males and females were collected.

(C) Probability of a bee being female in 38 years in which both males and females were collected and any bee was collected before or on the predicted flowering date.

Parentheses above the bars in (A) and (C) refer to number of years of collection; parentheses above the bars in (B) refer to the combined number of male and female collections, on or before predicted flowering date, within the temperature range.

12. Badeck, F.W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J., and Sitch, S. (2004). Responses of spring phenology to climate change. *New Phytol.* 162, 295–309.
13. Miller-Rushing, A.J., Inouye, D.W., and Primack, R.B. (2008). How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *J. Ecol.* 96, 1289–1296.
14. Panchen, Z.A., Primack, R.B., Anisko, T., and Lyons, R.E. (2012). Herbarium specimens, photographs, and field observations show Philadelphia area plants are responding to climate change. *Am. J. Bot.* 99, 751–756.
15. Gallagher, R.V., Hughes, L., and Leishman, M.R. (2009). Phenological trends among Australian alpine species: using herbarium records to identify climate-change indicators. *Aust. J. Bot.* 57, 1–9.
16. Primack, D., Imbres, C., Primack, R.B., Miller-Rushing, A.J., and Del Tredici, P. (2004). Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *Am. J. Bot.* 91, 1260–1264.
17. Lavoie, C., and Lachance, D. (2006). A new herbarium-based method for reconstructing the phenology of plant species across large areas. *Am. J. Bot.* 93, 512–516.
18. Calinger, K.M., Queenborough, S., and Curtis, P.S. (2013). Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. *Ecol. Lett.* 16, 1037–1044.
19. Memmott, J., Craze, P.G., Waser, N.M., and Price, M.V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecol. Lett.* 10, 710–717.
20. Bartomeus, I., Ascher, J.S., Wagner, D., Danforth, B.N., Colla, S., Kornbluth, S., and Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proc. Natl. Acad. Sci. USA* 108, 20645–20649.
21. Rafferty, N.E., and Ives, A.R. (2011). Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecol. Lett.* 14, 69–74.
22. Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.-L., and Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecol. Lett.* 12, 184–195.
23. Iler, A.M., Inouye, D.W., Høye, T.T., Miller-Rushing, A.J., Burkle, L.A., and Johnston, E.B. (2013). Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. *Glob. Change Biol.* 19, 2348–2359.
24. Schiestl, F.P., Ayasse, M., Paulus, H.F., Löfstedt, C., Hansson, B.S., Ibarra, F., and Francke, W. (2000). Sex pheromone mimicry in the early spider orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 186, 567–574.
25. Tremblay, R.L., Ackerman, J.D., Zimmerman, J.K., and Calvo, R.N. (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biol. J. Linn. Soc. Lond.* 84, 1–54.
26. Hutchings, M.J. (2010). The population biology of the early spider orchid, *Ophrys sphegodes* Mill III. Demography across three decades. *J. Ecol.* 98, 867–878.
27. Parker, D.E., Legg, T.P., and Folland, C.K. (1992). A new daily Central England temperature series, 1772–1991. *Int. J. Climatol.* 12, 317–342.